DATA EVALUATION RECORD

STUDY TYPE: REVIEW OF DEVELOPMENTAL NEUROTOXICITY STUDY
POSITIVE CONTROL DATA FROM BAYER CORPORATION

MRID 45441302

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Work Assignment No. 131-2006

Primary Reviewer: Virginia A. Dobozy, V.M.D., M.P.H	Signature: Robert H. Ross Date: APR 13 2006
Secondary Reviewers: Carol S. Wood, Ph.D., D.A.B.T.	Signature: Carol S Wood Date: APR 13 2006
Robert H. Ross, M.S. Group Leader	Signature: Robert H. Ross Date: APR 13 2006
Quality Assurance: Lee Ann Wilson, M.A.	Signature: L.A. Wilson Date: APR 13 2006

Disclaimer

This review may have been altered subsequent to the contractor=s signatures above.

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC., for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

EPA Reviewer: Marion Copley, D.V.M., D.A.B.T. **Signature**: _____
Science Information Mgmt. Branch, HED (7509P) **Date:** _____
EPA Secondary Reviewer: Jess Rowland, M.S. **Signature**: _____
Immediate Office, Health Effects Division (7509P) **Date:** _____

Template version 02/06

TXR#: 0054595

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; Positive Control, Non-guideline

PC CODE: 600093 **DP BARCODES:** D302810

TEST MATERIAL (PURITY): Methimazole (99.9% ai)

SYNONYMS: 2-Mercapto-1-methylimidazole

CITATION: Sheets L. P. (2001) Method validation study for a developmental neurotoxicity screen: Untreated (normative) and perinatal methimazole treatment in Wistar rats. Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS. Study Number 98-982-RR, February 9, 2001. MRID 45441302. Unpublished .

SPONSOR: Bayer Corporation

EXECUTIVE SUMMARY: In a study (MRID 45441302) designed to demonstrate the proficiency of Bayer Corporation to perform developmental neurotoxicity studies, methimazole (99.9% a.i., batch #097H3432) was administered via the drinking water to Wistar Crl:WI(HAN)BR rats (approximately 25/dose level) at nominal concentrations of 0 and 0.1 mg/mL from gestation day (GD) 16 through lactation day (LD) 10. Mean dose to the animals during GD 16-22, and lactation days (LD) 1-7 and 7-10 were 3.8, 5.1, and 6.0 mg/kg/day, respectively. Thyroid function was evaluated on some groups. An abbreviated Functional Observational Battery (FOB) was performed on 30 dams per group on GDs 7 and 14 and on 21-23 dams on LDs 7 and 14. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (abbreviated FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (passive avoidance and water maze testing) and neuropathology at termination (PND 70). On PND 11, the whole brain was collected from 10 pups/sex/group for micropathologic examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed. Thyroid function was evaluated on PND 11 and 70 (T_3, T_4 and thyroid receptor expression).

No maternal deaths or clinical signs of toxicity were observed. Body weight was significantly or slightly decreased in treated dams on GD 20 (94% of control value) and on lactation days 0-14 (94-97% of control value). Overall body weight gain during gestation (GDs 0-20) was significantly decreased (86% of control value) in treated dams. Food consumption during the

end of the gestation period (GDs 13-20) was significantly decreased (93% of control value) in treated dams. Food consumption during lactation was decreased (77-94% of control value) in treated dams but the effect was significant only for LDs 7-14. Water consumption was decreased (75-86% of control value) during the dosing period but the effect was significant only for LDs 7-10.

No treatment-related effects were observed in an abbreviated FOB in dams. Reproductive performance was not affected by treatment and there were no effects on litter size and viability.

On PND 0, there were no treatment-related effects on body weight of offspring. From PNDs 7 to 21, statistically significant decreases in body weight were observed in treated males (87-92% of control value) and females (87-91% of control value). Body weight gain was significantly decreased throughout most of the pre-weaning period in males (85-88% of control value) and females (83-91% of control value). Offspring body weight continued to be significantly decreased in treated males (88-96% of control value) and females (85-95% of control value) during the post-weaning period. Food consumption was significantly decreased in males (89-93% of control value) and females (89% of control value) post-weaning. In pups from treated dams, preputial separation in males was delayed by 1.7 days, vaginal opening in females was delayed by 4.1 days, and acquisition of acoustic startle reflex was delayed by 2.9 days (both sexes combined).

No treatment-related effects were observed on FOB parameters in offspring. On PND 13, motor activity was 43-46% of the control level and locomotor activity was 17-23% of control level in treated male and female offspring. Peak amplitude of acoustic startle response was significantly increased in treated males (140-147% of controls) and slightly increased in females (115-119% of controls) on PNDs 38 and 60. Latency was not affected by treatment at any of the testing periods. No treatment-related effects were observed on passive avoidance testing. The number of trials to criterion during both the learning and retention phases of the water maze testing was higher for treated animals with the change being significant in males during the learning phase. Trial duration of trials one and two was also increased in treated animals; the change was significant in males during the learning phase.

Anti-thyroid effects of the chemical were demonstrated as decreased T_4 and T_3 levels and increased incidence of hypertrophy/hyperplasia at the PND 11 necropsy.

Absolute brain weight was decreased (91-92% of control value) in treated male and female offspring on PND 11 but was unaffected by treatment on PND 70. On microscopic examination, the incidence and/or severity of oligodendroglia prominence and neuropil maturation was increased in treated male and female offspring in most brain sections. Morphometric measurements in the frontal cortex, parietal cortex, caudate putamen and hippocampal gyrus were significantly decreased in treated males on PND 11.

This study is classified **Acceptable/Non-guideline (except for FOB). Bayer Corporation has demonstrated proficiency in this study for detecting changes in Auditory Startle, Motor Activity, Thyroid Function, and Brain Neuropathology and Morphometry in pre- and postweaning and young adult (depending on endpoint) Wistar Crl:WI(HAN)BR rats due to methimazole treatment for the time period around 1998-1999 (in life period of study). This positive control study does not satisfy any guideline requirement.** FOB data presented in this study is inadequate to determine whether the laboratory has proficiency for detecting changes in this parameter.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

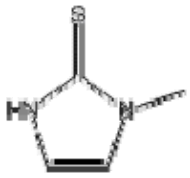
I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Methimazole

Description:	Off-white powder
Lot/batch #:	097H3432
Purity:	99.9% a.i.
Compound stability:	Not provided
CAS # of TGAI:	60-56-0

Structure:



2. Vehicle: Drinking water

3. Test animals (P):

Species:	Rat
Strain:	Wistar Crl:W(HAN)BR
Age at study initiation:	Males: 15wks; females: 12 weeks
Wt. at study initiation:	Females – approximately 225±20% g
Source:	Charles River Laboratories Inc., Margate Kent, UK
Housing:	Males and females in individual stainless steel cages, except during co-habituation; individual dams and litters in plastic cages during gestation and lactation; littermates together in plastic cages until PND 28, then individually in stainless steel cages
Diet:	Purina Mills Rodent Lab Chow 5001-4 *ad libitum*
Water:	*ad libitum*; Tap water until GD 14, then deionized water
Environmental conditions:	Temperature: 19-25ºC Humidity: 30-70% Air changes: Not provided Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	Six days

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: November 11, 1998; End: January 29, 1999

2. Study schedule: Mated female Wistar rats (30/dose group) were administered the test material in the water from gestation day (GD) 16 through lactation day (LD) 10. On postnatal day (PND) 4, litters were standardized to 8 pups; sexes were represented as equally as possible. Pups were weaned from their dam on PND 21 but were not treated with test material. Dams were sacrificed after weaning. Pups remained on study to PND 70.

3. **Mating procedure:** One resident sexually mature male and one female were co-housed for a maximum of five days. The day that a vaginal plug or sperm in a vaginal smear was observed was designated gestation day (GD) 0. Each pregnant female was placed into a plastic nesting cage, where it was maintained through gestation and lactation.
4. **Animal assignment**: After the acclimation period, dams with body weight more or less than 20% of the mean weight were rejected and sacrificed without necropsy. The remaining females were assigned to the control or treated groups in sequence as they were inseminated. Parental generation males were only breeders and were arbitrarily selected for co-housing with females. Offspring were randomly assigned to testing subgroups at the time of litter standardization on PND 4 (Table 1).

TABLE 1. Study design

Experimental parameter	Water concentration (mg/mL)	
	0	0.1
Maternal animals		
No. of maternal animals assigned		
No. of maternal animals assigned	30	30
Abbreviated FOB (GDs 6, 10, 15, 20)	30	30
Abbreviated FOB (LDs 4, 11, 21)	23	21-22
Offspring		
Minimum No. of offspring assigned		
Set A - motor activity (PNDs 13, 17, 21 and 60±2) Tissues, brain weight (PND 70±7)	20-25/sex 6/sex	20-25/sex 6/sex
Set B - Acoustic startle habituation (PNDs 22, 38±2 and 60±2) Thyroid analyses (PND 70±7)	20-25/sex 10/sex	20-25/sex 10/sex
Set C - Passive Avoidance (PNDs 24, 31), Water Maze (PND 60±2, 67±2) Abbreviated FOB (PNDs 4, 11, 21, 35, 45, 60)	20-25/sex 15/sex	20-25/sex 15/sex
Set D – Tissues, brain weight (PND 11) Thyroid analyses (PND 70)	20-25/sex 10/sex	20-25/sex 10/sex

5. **Dose selection rationale:** The basis for the dose levels was a published study in which Sprague-Dawley rats received methimazole in the drinking water at a concentration of 0.1 mg/mL from GD 16 through LD 10. Developmental delays and motor deficits were observed in offspring, including delays in righting response, appearance of the auditory startle response and the age of eye opening. Decreases in body weight gain and exploratory activity in the open field were also observed at PND 21.

6. **Dosage administration:** Beginning on GD 14, access to the automatic watering system was withdrawn from all dams and deionized water in a bottle with a drinking tube was the only source of drinking water. On GD 16, water bottles of treated females were replaced with bottles containing methimazole (0.1 mg/mL). Fresh solutions were provided as needed through LD 10 and clean bottles were provided weekly.

7. **Dosage preparation and analysis:** No details on the preparation of the dosing solutions were provided. The stability (following both room temperature and freezer exposure) was

established prior to the start of the study. No results for homogeneity, stability or concentration analyses were provided. However, the report stated that the methimazole concentration averaged 0.1 mg/mL throughout the exposure period to the dams.

No analytical data were submitted to demonstrate that the mixing procedure was adequate and that the difference between nominal and actual dosage to the animals was acceptable.

C. OBSERVATIONS:

1. In-life observations:

a. Maternal animals: Parental generation females were observed cage-side for mortality, moribundity and clinical signs of toxicity at least once daily during the study. A physical examination of females was performed once daily during the dosing period. Females presumed to be pregnant (approximately 30 per group) were observed outside the home cage for an abbreviated functional observational battery (FOB) four times during the gestation period (days 6, 10, 15, 20). Dams which delivered and were acceptable for study use (21-23/group) were examined three times during the lactation period (days 4, 11 and 21). The arena size and examination details were not provided. Most of the following functional observations were recorded.

Functional observations–Maternal animals	
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe; 2) Presence or absence of piloerection and exophthalamus; 3) Ranking or count of urination and defecation, including polyuria and diarrhea; 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size; 5) Degree of palpebral closure, e.g., ptosis; 6) Respiration; 7) Activity/arousal level.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Food consumption was measured weekly during gestation and lactation for the following periods: GDs 0-6, 6-13, 13-20 and LDs 0-7, 7-14 and 14-21. Water consumption and body weight were measured weekly during the exposure period (GD 16 – LD 10) in order to establish the dosage (mg/kg body weight/day) of methimazole and relative amounts of water consumed by control and treated dams. Body weight of dams was also recorded on LD 4, weekly during the non-exposure period of the study, and prior to sacrifice.

b. Offspring:

1.) Litter observations: The day of completion of parturition was designated as PND 0. The number of pups delivered and the pup status at birth were recorded for each litter. If

a dam delivered fewer than three pups per sex or if the litter size decreased to less than seven pups by PND 11, the dam and litter were sacrificed without necropsy. Observations for clinical signs were made daily during the pre-weaning period.

On PND 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible) using a random selection technique. Pups were weighed on PNDs 4, 7, 14, 21 and weekly thereafter, until termination.

2.) **Developmental landmarks**: Pups were evaluated daily for the following developmental landmarks and reflex-based behavior as follows: pinnae detachment (PND 1), surface righting (PND 4), auditory startle (PND 10) and eye opening (PND 11). Sexual maturation was evaluated by inspecting females daily for vaginal patency beginning on PND 29 and males for preputial separation beginning on PND 38. Pupil constriction was tested on PND 21.

3.) **Postweaning observations:** After weaning on postnatal day 21, offspring were examined once weekly for clinical signs. Body weight was measured weekly. Food consumption was measured weekly from the time the first litter reached PND 28 (beginning of their placement into single housing).

4.) **Neurobehavioral evaluations**: Observations and the schedule for those observations are summarized as follows from the report

i. **Functional observational battery (FOB) (Set C):** On PNDs 4, 11, 21, 35, 45, and 60, 15 offspring/dose level assigned to Set C were examined outside the home cage in an abbreviated FOB. The same parameters assessed in the maternal FOB were examined in offspring, as appropriate for the developmental stage being observed.

ii. **Motor activity testing (Set A):** Motor activity was evaluated in one male and one female from each litter/group on PNDs 13, 17, 21 and 60±2. Activity was monitored for 60 minutes (six ten-minute intervals) in figure-eight mazes. Each maze consisted of eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys). A Columbus Instruments Universal Maze Monitoring System was used for data collection. Broad-spectrum background noise was provided throughout the test to minimize acoustical variation during testing. The uniformity of light intensity over each maze was verified each day. Motor activity was measured as the number of beam interruptions that occurred during each session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of each session.

iii. **Auditory startle reflex habituation (Set B):** Auditory startle reflex habituation testing was performed on one male and one female/litter/group on PNDs 22, 38±2 and 60±2. Groups of four animals were tested simultaneously within the startle system enclosure. The enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material and housed a speaker mounted in the ceiling to

provide the eliciting stimulus (a 40-msec burst of white noise at approximately 120 dB). The enclosure housed four load cell/force transducer assemblies that measured the startle response. During the test session, the animals were placed in cages that were positioned on the top of each load cell. Sound measurements were made using a Bruel and Kjaer Real-Time Frequency Analyzer fitted with a microphone. The animals were allowed a 5-minute acclimation period in the enclosure at ambient noise levels before being presented with the startle-eliciting stimulus at 10-sec intervals. Data collection began with the presentation of the stimulus and continued for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (one sample/msec for 200 msec) and converted to grams using a calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's response curve. Baseline was defined as the average force (g) exerted on the platform during the first six msec following the onset of the stimulus. This baseline was taken to represent an approximate body weight measurement to verify that the equipment was functioning properly. Response amplitude was defined as the maximum value of the average curve minus the baseline. Latency to peak was the time (msec) following the onset of the stimulus when the peak response occurred.

iv. **Learning and memory testing (Set C):** Learning, short-term retention and long-term retention were assessed in a **passive avoidance test** on PNDs 24 and 31. One male and one female/litter/group were tested. Only animals that demonstrated acquisition were tested for retention. Testing was conducted using an integrated system of equipment and computer programs from Coulbourn Instruments. Testing occurred in individual isolation cubicles, each with a single shuttle cage. Each shuttle cage was separated into two compartments of equal size by a wall that supported a centrally-located sliding door. The walls of one compartment were lined with black film (dark side) and the walls of the other compartment were illuminated with a high-intensity lamp. After adaptation, the animal was placed into the lighted compartment facing toward the light. After approximately 20 seconds, the light was illuminated and the door between the compartments was opened. When the rat moved into the dark compartment, the door closed, a shock was delivered and the light was switched off. The rat was then returned to its cage until the next trial. If the rat did not cross to the dark compartment within 180 seconds, it was returned to its cage and given a latency score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on two consecutive trials or until 15 trials elapsed, whichever occurred first. Animals that failed to reach criterion performance with 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase. The test was repeated one week later. In the second trial, rats were placed in the illuminated compartment, given a 20-second acclimation period and then the latency to enter the dark side was recorded. The dependent measures were the number of trials to criterion, latency to cross on Trial 1 and Trial 2 (learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Learning and memory were also assessed in a minimum of 16 animals/sex/group using **water maze testing** on PND 60±2 and again seven days later. The M-maze was constructed of Plexiglas with five inch wide corridors and contained approximately 7.5 inches of water maintained at 22±1ºC. For each trial, the rat was placed in the starting position at the base of the M-maze stem, located between the two lateral arms. For the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the maze. The initial arm for the learning trial was the incorrect goal for the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any trial were guided to the correct goal with the exit ramp and removed from the maze. Between trials, the animals were kept in a transport cage for approximately 15±5 seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in a test session was fifteen. Latency (time in seconds to choose the correct goal or the maximum of 60 seconds) and the number of errors (incorrect turns in the maze) were recorded. Only animals that demonstrated acquisition within the 15-trial limit were tested for retention seven days later. Dose groups were compared using the following measures:

Acquisition (First Test): number of trials to criterion (measure of overall learning); average number of incorrect turns in maze for each trial (measure of overall learning); and latency to reach the correct goal on trial 2 (measure of short-term retention).

Retention (Second Test): number of trials to criterion (measure of long-term retention); average number of errors for each trial (measure of long-term retention); and latency to reach the correct goal on trial 1 (measure of long-term retention).

5) **Ophthalmology**: Pre-terminal ophthalmic examinations were conducted on rats selected for perfusion at study termination. Pupillary reflex was tested using a penlight or transilluminator after dilation of the pupils with a mydriatic. The eyelid, conjunctiva, cornea, aqueous humor and lens were examined with a slit lamp microscope either before or after pupillary dilation. After dilation, the vitreous humor, retina, choroid and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

6) **Clinical Pathology (Sets B and D)**: At the PND 11 necropsy, blood samples were collected via decapitation from half of the pups (10/sex/group) in Set D for measurement of T_3 and T_4 levels. On PND 70±7, blood was collected from the orbital plexus from half of the pups (10/sex/group) in Set B for T_3 and T_4 levels. In addition, thyroid tissue from a minimum of five males and five females per dose group (day 11 pups and day 70±7 animals) was stained for thyroid receptor expression and evaluated microscopically.

2. Postmortem observations:

a. Maternal animals: Following co-habitation, males were sacrificed by CO_2 asphyxiation and discarded. Dams were sacrificed by CO_2 asphyxiation on LD 21 after the weaning of their litters; necropsy was not conducted. Mated females that did not deliver a litter were sacrificed on GD 24 without necropsy.

b. Offspring: All moribund pups were sacrificed and subjected to gross necropsy. Tissues were collected at the discretion of the study director. Animals found dead underwent necropsy and were disposed of without collection of tissues. Pups selected for culling were sacrificed by injection of Fatal Plus and discarded without necropsy.

On PND 11, all pups assigned to Set D underwent gross necropsy. Approximately half of the pups allocated to Set D were sacrificed via intraperitoneal injection of Fatal Plus. Thyroid tissue was removed and fixed in buffered 10% formalin. If available, one additional male and female from each litter of Set D were euthanized by severing the head at the base of the skull. After collection of blood for the thyroid function measures, the calvaria was sliced to expose the brain and the entire head was immersed in buffered 10% formalin. After fixation, the brain with olfactory bulbs was removed and weighed. Prior to histological processing, the following two linear measurements (mm) were made: 1) anterior-to-posterior length of the cerebrum, extending from the anterior pole to posterior pole, exclusive of the olfactory bulbs; and 2) anterior-to-posterior length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole. These measurements were performed by a technician who was aware of the dose assignments. Each brain was then divided into eight coronal sections for microscopic examination. The sections were processed for paraffin embedding, sectioned and examined using hematoxylin and eosin (H & E), luxol fast blue/cresyl violet and Sevier-Munger stains.

Nine linear measurements were taken, including the two gross measures of the intact brain (cerebrum and cerebellum). The other seven measures are as follows:

1. Frontal cortex thickness (Level 4) - measurement of the dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm.

2. Parietal cortex thickness (Level 4) - measurement of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.

3. Caudate putamen and underlying globus pallidus diagonal width (Level 4; maximum cross-sectional width) - measurement on the coronal section at the level of the optic chiasm.

4. Corpus callosum thickness (Level 4) – measurement taken at the mid-point of this brain region, within the section taken at the level of the optic chiasm.

5. Hippocampal gyrus thickness (Level 5) - measurement of the full width of the hippocampal gyrus from the ventral tail of the dentate gyrus to the overlying subcortical white matter. Measurements were taken from the hippocampus from both sides and the mean value was recorded.

6. Cerebellum height (Level 7) - measurement extending from the roof of the fourth ventricle to the dorsal surface.

7. External germinal layer (Level 7) – multiple areas were measured over the dorsum of the cerebellum and the mean value recorded as one measurement.

At the **PND 70±5** necropsy, the following procedures were followed for the different sets of animals:

Set C: Sacrificed by carbon dioxide asphyxiation without routine collection of tissues.

Set B: Sacrificed by carbon dioxide asphyxiation. Thyroid removed, weighed and fixed in buffered 10% formalin.

Set A: The first six males and females per group were selected for perfusion and collection of neural tissues. The animals were deeply anesthetized using an intraperitoneal injection of pentobarbital and were then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by *in situ* fixation using 10% buffered formalin. The entire brain and spinal cord, both eyes (with optic nerves), selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs and a physical identifier were collected from each animal and post-fixed in 10% buffered formalin. The brain was weighed prior to placement in the fixative. Measurements of the cerebrum and cerebellum were made as described under PND 11. The brains from this set were used for microscopic evaluation. Eight coronal sections of the brain and sections from four levels of the spinal cord (cervical, thoracic, lumbar and cauda equina) were embedded in paraffin and examined using H&E, luxol fast blue/cresyl violet and Sevier-Munger stains. The thyroid was processed using standard procedures for paraffin embedding, sectioned and stained with H&E. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings, gasserian ganglia, eyes, optic nerves and gastrocnemius muscle were embedded in glycol methacrylate (GMA), sectioned and stained with modified Lee's stain. Peripheral nerves (sciatic, tibial and sural) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section. Eight linear measurements, as described under PND 11, were made. The remaining animals in Set A were sacrificed by carbon dioxide asphyxiation and necropsied but tissues were not collected.

D. DATA ANALYSIS:

1. **Statistical analyses:** Continuous data were assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. If there were unequal variances, the data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons).

Motor and locomotor activity (total session activity and activity for each 10-minute interval) were analyzed using ANOVA procedures. Session activity data for the four test occasions were analyzed using an ANOVA to determine if there was a significant day-by-treatment interaction. If so, Dunnett's test was used to determine if the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine if there was a significant treatment-by-interval interaction on each test occasion. If so, the data were analyzed using Dunnett's test to determine whether the treated group was significantly different from the control.

Acoustic startle response amplitude data (peak amplitude) for the first three occasions were first analyzed using an ANOVA. If there was a group effect, Dunnett's test was used to determine if the treated group was significantly different from the control. The response amplitude data for each block of ten trials (five blocks/test session) were analyzed using a Repeated-Measures ANOVA, using test block as the repeated measure. If there was a significant group-by-block interaction, the block values were analyzed using Dunnett's test to determine if the treated group was significantly different from the control.

Passive avoidance and water maze data were analyzed using a univariate ANOVA with post-hoc analysis using the Dunnett's test. Latency data were analyzed using parametric statistical procedures. Trials to criterion measures were analyzed using nonparametric tests. The number of rats failing to meet the criterion level of performance in the learning phase was analyzed as incidence data.

Micropathology frequency data were screened for potential effects and then evaluated using a Chi-Square procedure, followed by a one-tailed Fischer's Exact Test in cases of significant variation by this analysis.

These methods are appropriate for the parameters tested.

2. <u>Indices</u>:

a. <u>Reproductive indices</u>: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

$$\text{Mating index (\%)} = \frac{\text{number of inseminated females}}{\text{number of females cohoused with males}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{number of pregnant females}}{\text{number of inseminated females}} \times 100$$

b. <u>Offspring viability indices</u>: The following viability (survival) indices were calculated from lactation records of litters in the study:

$$\text{Live birth index (\%)} = \frac{\text{number of live pups born per litter}}{} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{number of live pups on Day 4 pre-culling per litter}}{\text{total number of pups per litter}} \times 100$$

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No deaths or clinical signs of toxicity were reported in dams during gestation or lactation. No treatment-related effects were observed during the abbreviated FOB on GDs 6, 10, 15, 20 and LDs 4, 11 and 21.

2. **Body weight, food and water consumption:** Selected group mean body weight, food consumption and water consumption data for pregnant and nursing dams are summarized in Table 2. Body weight was significantly decreased (94% of control value) in treated dams on GD 20. Overall body weight gain during gestation (GDs 0-20) was significantly decreased (86% of control value) in treated dams. Food consumption during the end of the gestation period (GDs 13-20) was significantly decreased (93% of control value) in treated dams. Body weight was significantly but minimally decreased (94-97% of control value) in treated females during the first 14 days of lactation. Food consumption was decreased (77-94% of control value) in treated dams during lactation but the effect was significant only for LDs 7-14. Water consumption was decreased (75-86% of control value) during the dosing period but the effect was significant only at the end of treatment.

TABLE 2. Mean (±SD) maternal body weight, food consumption and water consumption [a]		
	Water concentration (mg/mL)	
	0	0.1
Observations/study day		
Gestation		
Mean body weight (g) Gestation day 0	233.3 ± 2.16	228.6 ± 2.25
Mean body weight (g) Gestation day 13	268.9 ± 3.58	266.4 ± 2.80
Mean body weight (g) Gestation day 20	339.8 ± 4.09	320.5** ± 4.55 (94)[b]
Mean weight gain (g) Gestation days 0-20	106.5 ± 2.58	91.9** ± 3.00 (86)
Mean food consumption (g/kg/day) Gestation days 0-6	80.5 ± 4.06	71.7 ± 3.54
Mean food consumption (g/kg/day) Gestation days 13-20	85.9 ± 1.33	80.0** ± 1.40 (93)
Mean water consumption (g/animal/day) Gestation days 16-22/Lactation day 0 (±2)	40.1 ± 14.2	34.4 ± 13.2 (86)
Lactation		
Mean body weight (g) Lactation day 0	267.3 ± 2.9	256.4* ± 3.2 (96)
Mean body weight (g) Lactation day 21	291.2 ± 6.0	295.9 ± 3.1
Mean weight gain (g) Lactation days 0-20[c]	23.9	39.5
Mean food consumption (g/kg/day) Lactation days 0-7	149.7 ± 9.3	134.7 ± 9.2 (90)
Mean food consumption (g/kg/day) Lactation days 14-21	231.0 ± 15.6	218.2 ± 10.2 (94)
Mean water consumption (g/animal/day) Lactation day 10 (±2)	79.5 ± 12.4	59.7* ± 8.3 (75)

[a] Data obtained from pages 56-60 and 63-66, MRID 45441302.
[b] Number in parentheses is percent of control calculated by reviewer.
[c] Calculated by the reviewer without standard deviation.
** Statistically significantly different from control, p≤ 0.01.
N = 29 during gestation and 21-23 during lactation.

3. **Test Substance Intake:** Based on the concentration of the test material (mg/mL) and water consumption (mL/animal/day), the amount of methimazole consumed by treated animals during late gestation (GD 16 through 22), the first week following parturition and the last three days of treatment (LDs 7-10) averaged 3.8, 5.1 and 6.0 mg/day, respectively.

4. **Reproductive performance:** Of the 30 females/group which were mated, 29 litters/group were born. The numbers of litters accepted for study use were 23 and 21 for the control and treated groups, respectively. The fertility index was 96.7% for both control and treated groups. The mean duration of gestation was 21.8 and 21.9 days for the control and treated rats, respectively. Results for the maternal animals are summarized in Table 3.

TABLE 3. Reproductive performance [a]		
	Water concentration (mg/mL)	
	0	0.1
Observation		
Number mated	30	30
Number of litters[b]	23	21
Mating index (%)	100.0	100.0
Fertility index (%)	96.7	96.7
Intercurrent deaths	0	0
Mean (±SE) gestation duration (days)	21.8 ± 0.12	21.9 ± 0.14
Incidence of dystocia	0	0

[a] Data obtained from page 52, MRID 45441302.
[b] Number of litters accepted for study use (litters with less than three pups/sex or litter size less than seven were sacrificed).

B. OFFSPRING:

1. **Viability and clinical signs**: Litter size and viability (survival) during lactation are summarized in Table 4. The mean number of pups delivered per dam and the percentage of liveborn and stillborn pups were not affected by treatment. There was no treatment-related effect on sex ratio on the day of birth or PND 21. No treatment-related clinical signs of toxicity were observed.

TABLE 4. Litter size and viability [a]		
	Water concentration (mg/mL)	
	0	0.1
Observation		
Number of litters[b]	23	21
Total number of pups born	271	233
Total number of pups missing	2	3
Total number of pups found dead	4	2
Number of stillborn pups	1	0
Total number of pups cannibalized	0	0
Mean litter size (±SE)	11.8 ± 0.38	11.1 ± 0.34
Sex Ratio Day 0 (% male) (±SE)	50.5 ± 2.53	49.7 ± 3.15

Mean No. of Viable Pups/Litter		
- Birth	12	11
- Day 4 (Precull)	12	11
- Day 4 (Postcull)	8	8
- Day 21	6	6
Live birth index (%) (±SE)	99.6 ± 0.36	100.0 ± 0.00
Viability index (%) (±SE)	99.0 ± 0.55	98.7 ± 0.70

[a] Data obtained from pages 70-71, MRID 45441302.
[b] Number of litters accepted for study use (litters with less than three pups/sex or litter size less than seven were sacrificed).

2. **Body weight**: On PND 0, there were no treatment-related effects on body weight. From PNDs 7 to 21, statistically significant decreases were observed in body weight of treated males (87-92% of control value) and females (87-91% of control value). Body weight gain was significantly decreased throughout most of the pre-weaning period in males (85-88% of control value) and females (83-91% of control value). Selected mean preweaning pup body weight and body weight gain data are presented in Table 5.

TABLE 5. Selected mean (±SE) pre-weaning pup body weight and body weight gain [a]

	Water concentration (mg/mL)			
	0	0.1	0	0.1
PND	Males		Females	
Body Weight (g)				
0	6.0 ± 0.08	5.9 ± 0.10	5.7 ± 0.08	5.6 ± 0.10
4 (pre-cull)	9.3 ± 0.23	8.8 ± 0.23 (95)[b]	9.0 ± 0.22	8.5 ± 0.22 (94)
4 (post-cull)	9.3 ± 0.24	8.8 ± 0.23 (95)	9.0 ± 0.22	8.4 ± 0.23 (93)
7	14.2 ± 0.36	13.0* ± 0.32 (92)	13.8 ± 0.32	12.5** ± 0.32 (91)
14	29.5 ± 0.55	25.8** ± 0.71 (87)	28.9 ± 0.54	25.0** ± 0.63 (87)
21	46.0 ± 0.97	41.1** ± 1.00 (89)	44.4 ± 0.93	39.3** ± 0.84 (89)
Body Weight Gain (g)				
0-4	3.3 ± 0.17	2.9 ± 0.17 (88)	3.2 ± 0.15	2.9 ± 0.14 (91)
4-7	4.9 ± 0.16	4.2** ± 0.14 (86)	4.9 ± 0.13	4.1** ± 0.13 (84)
7-14	15.2 ± 0.32	12.9** ± 0.51 (85)	15.0 ± 0.32	12.5** ± 0.48 (83)
4-21	36.7 ± 0.79	32.3** ± 0.87 (88)	35.4 ± 0.78	30.9** ± 0.72 (87)

[a] Data obtained from pages 77-83, MRID 45441302.
[b] Number in parentheses is percent of control; calculated by reviewer.
PND = post-natal day
N = 21-23
* Statistically significantly different from control, p≤ 0.05
** Statistically significantly different from control, p≤0.01

Offspring body weight continued to be significantly decreased in treated males (88-96% of control value) and females (85-95% of control value) during the post-weaning period. Food consumption was significantly decreased in males during the first three weeks post-weaning

(89-93% of control value) and in females (89% of control value) for the first week post-weaning. Selected mean postweaning offspring body weight and food consumption data are presented in Table 6.

Table 6. Mean (±SD) post-weaning pup body weight (g) [a]

PND	Water concentration (mg/mL)			
	Males		Females	
	0	0.1	0	0.1
Body Weight (g)				
28	70.6 ± 8.9	61.8* ± 8.2 (88)[b]	65.8 ± 6.7	56.1* ± 7.2 (85)
35	116.9 ± 13.7	103.5* ± 11.7 (89)	104.3 ± 9.2	92.6* ± 8.9 (89)
42	164.2 ± 18.2	150.0* ± 12.8 (91)	135.3 ± 10.6	124.6* ± 8.3 (92)
70	318.3 ± 27.9	305.4* ± 22.9 (96)	199.9 ± 16.2	197.6 ± 12.5
Food consumption (g/animal/day)				
28-35	16.57 ± 2.47	14.78* ± 1.61 (89)	15.97 ± 2.89	14.16* ± 1.74 (89)
35-42	24.26 ± 3.38	22.51* ± 2.98 (93)	24.53 ± 6.01	26.37 ± 9.85
63-70	26.49 ± 2.07	25.89 ± 2.17	21.18 ± 3.15	20.55 ± 3.76

[a] Data obtained from pages 87-89, MRID 45441302.
[b] Number in parentheses is percent of control; calculated by reviewer.
PND = post-natal day
N = 21-23
* Statistically significantly different from control $p \leq 0.05$

3. Developmental landmarks:

a. Sexual maturation: Preputial separation in males and vaginal opening in females were significantly delayed in treated animals. The data are presented in Table 7. Body weight at attainment was not reported.

TABLE 7. Mean (±SD) age of developmental landmarks (days) [a]

Parameter	Water concentration (0.1 mg/mL)			
	Males		Females	
	0	0.1	0	0.1
Number of litters	23	21	23	21
Preputial separation	44.9 ± 0.44	46.6** ± 0.40	-	-
Vaginal opening	-	-	33.1 ± 0.49	37.2** ± 0.40

[a] Data obtained from pages 85, MRID 45441302.
** Statistically significantly different from control, $p \leq 0.01$.

b. Other developmental landmarks: Acquisition of acoustic startle response was delayed in the combined sexes (16.0±0.16 in treated animals vs. 13.1±0.13 in the control group). Other developmental landmarks were not affected by treatment.

4. Behavioral assessments:

a. Functional observational battery: No treatment-related findings were observed at any of the testing periods. Significant findings in treated groups were sporadic.

b. Motor activity: Total motor activity data are presented in Table 8a. On PND 13, total motor activity was decreased in treated males and females (43-46% of control value). Total locomotor activity was also decreased in treated males and females (17-23% of control value) on PND 13. Motor and locomotor activity in treated animals was comparable to the control group on PNDs 17, 21, and 60. A significantly greater locomotor activity in treated females on PND 60 is probably due to normal variation as no consistent effect or trend was noted in the subsession data.

Motor activity interval data are included in Table 8b. On PND 13, motor activity was decreased at all six intervals of the sessions in males (29-55% of control value) and females (24-68% of control value). On PND 60, decreased activity was observed on 5/6 intervals in treated males (83-92% of control value) and females (70-90% of control value). Locomotor activity interval data are included in Table 8c. Decreases in activity were observed in treated males and females at all intervals on PND 13. On PND 17, no treatment-related effects were observed. On PND 21, a few increases in activity were recorded in treated males and females; whereas, significant decreases in activity were reported in females on PND 60.

Habituation of motor activity was evident in control and treated animals at all four testing periods. Locomotor activity habituation was evident on PNDs 17, 21 and 60 but was too low to assess on PND 13.

TABLE 8a. Mean (±SD) motor and locomotor activity data (total activity counts for session) [a]

Test Day	Water concentration (mg/mL)			
	Males		Females	
	0	0.1	0	0.1
Motor activity				
PND 13	205 ± 194	88 ± 76 (43)[b]	191 ± 162	87 ± 89 (46)
PND 17	495 ± 220	488 ± 254	466 ± 180	466 ± 264
PND 21	553 ± 211	544 ± 250	519 ± 182	564 ± 200
PND 60	702 ± 176	646 ± 161	929 ± 305	772 ± 290
Locomotor activity				
PND 13	23 ± 31	4 ± 5 (17)	22 ± 28	5* ± 13 (23)
PND 17	100 ± 66	101 ± 73	100 ± 49	104 ± 60
PND 21	107 ± 43	119 ± 49	106 ± 42	135* ± 40 (127)
PND 60	367 ± 82	372 ± 83	449 ± 123	367 ± 166

[a] Data obtained from pages 193-197, MRID 45441302.

[b] Number in parentheses is percent of control; calculated by reviewer.

N = 21-23

** Statistically significantly different from control, ($p \leq 0.05$, ANOVA).

TABLE 8b. Motor activity sub-sessions (# movements/10 minute period ±SD) [a]

Sub-session		Water concentration (mg/mL)			
		Males		Females	
		0	0.1	0	0.1
PND 13	1	61 ± 71	23* ± 32 (38)[b]	54 ± 57	23 ± 46 (43)
	2	44 ± 48	18* ± 27 (41)	34 ± 36	15 ± 31 (44)
	3	31 ± 39	16 ± 17 (52)	40 ± 38	17* ± 24 (43)
	4	23 ± 35	11 ± 15 (48)	22 ± 25	14 ± 20 (64)
	5	24 ± 43	7 ± 9 (29)	19 ± 31	13 ± 16 (68)
	6	22 ± 48	12 ± 17 (55)	21 ± 26	5* ± 10 (24)
PND 17	1	149 ± 63	168 ± 55	139 ± 56	164 ± 94
	2	77 ± 47	102 ± 51	81 ± 49	90 ± 65
	3	65 ± 50	77 ± 57	64 ± 50	67 ± 43
	4	65 ± 54	46 ± 54	61 ± 50	58 ± 52
	5	62 ± 49	55 ± 56	60 ± 52	48 ± 44
	6	78 ± 68	40* ± 52 (51)	61 ± 49	39 ± 42
PND 21	1	184 ± 68	180 ± 67	170 ± 44	187 ± 59
	2	106 ± 42	109 ± 55	102 ± 41	100 ± 34
	3	81 ± 43	89 ± 46	78 ± 34	93 ± 41
	4	73 ± 40	66 ± 37	65 ± 38	60 ± 39
	5	53 ± 46	47 ± 54	59 ± 47	65 ± 54
	6	56 ± 50	52 ± 55	45 ± 40	59 ± 48
PND 60	1	143 ± 48	147 ± 42	188 ± 71	190 ± 59
	2	124 ± 47	110 ± 32 (89)	153 ± 74	137 ± 64 (90)
	3	122 ± 37	112 ± 38 (92)	160 ± 58	118* ± 53 (74)
	4	110 ± 37	101 ± 30 (92)	153 ± 54	115* ± 53 (75)
	5	102 ± 30	85 ± 29 (83)	148 ± 49	103* ± 53 (70)
	6	102 ± 37	90 ± 36 (88)	127 ± 46	110 ± 53 (87)

[a] Data obtained from pages 199-206, MRID 45441302.

[b] Number in parentheses is percent of control calculated by reviewer.

N = 21-23

* Statistically different from control, $p<0.05$

TABLE 8c. Locomotor activity sub-sessions [# movements/10 minute interval ±SD] [a]					
Sub-session		Water concentration (mg/mL)			
		Males		Females	
		0	0.1	0	0.1
PND 13	1	5 ± 8	1* ± 1	4 ± 6	1 ± 2
	2	5 ± 10	0* ± 1	3 ± 6	1 ± 4
	3	4 ± 8	1 ± 2	5 ± 10	1 ± 3
	4	3 ± 5	0* ± 1	4 ± 7	1 ± 2
	5	4 ± 8	1 ± 1	3 ± 7	1 ± 1
	6	2 ± 5	1 ± 2	3 ± 6	1 ± 3
PND 17	1	27 ± 14	33 ± 16	29 ± 12	31 ± 16
	2	12 ± 11	20 ± 14	15 ± 11	19 ± 13
	3	12 ± 11	15 ± 16	12 ± 10	16 ± 12
	4	15 ± 16	10 ± 12	14 ± 14	15 ± 12
	5	14 ± 13	13 ± 16	14 ± 12	12 ± 13
	6	20 ± 20	10 ± 14	15 ± 15	11 ± 13
PND 21	1	43 ± 19	44 ± 15	40 ± 11	51* ± 11
	2	19 ± 7	21 ± 9	19 ± 8	24 ± 10
	3	14 ± 8	17 ± 10	14 ± 8	21* ± 9
	4	11 ± 7	17* ± 10	13 ± 8	13 ± 9
	5	9 ± 8	10 ± 11	11 ± 10	13 ± 11
	6	10 ± 11	11 ± 12	10 ± 10	12 ± 11
PND 60	1	76 ± 18	87 ± 20	84 ± 17	89 ± 31
	2	61 ± 29	63 ± 20	65 ± 31	62 ± 40
	3	65 ± 19	67 ± 26	81 ± 36	61 ± 32
	4	56 ± 21	57 ± 18	80 ± 31	55* ± 33
	5	54 ± 18	51 ± 21	76 ± 27	51* ± 33
	6	55 ± 24	48 ± 24	62 ± 24	50 ± 35

[a] Data obtained from pages 208-215, MRID 45441302.
N = 21-23
* Statistically different from control, $p<0.05$

c. <u>Auditory startle reflex habituation</u>: The overall peak amplitude and latency data are presented in Table 9a. No treatment-related effects were seen on PND 22. Peak amplitude was significantly increased (140-147% of control value) in treated males and non-significantly increased (115-119% of control value) in treated females on PNDs 38 and 60. Latency was not affected by treatment at any of the testing periods. Interval amplitude and latency data are included in Tables 9b (males) and 9c (females). During the intervals on PNDs 38 and 60, peak amplitude values were significantly increased in 3/5 and 5/5 blocks, respectively, in the treated males. In general, peak amplitude was increased in treated females on PNDs 38 and 60 but no significant effects were observed. Latency was unaffected by treatment in males and females. Habituation was apparent in control and treated animals on PNDs 38 and 60, but not on PND 22.

TABLE 9a. Mean (±SD) auditory startle peak amplitude (g) and latency to peak (msec) in F_1 male and female rats [a]

Parameter	Water concentration (mg/mL)			
	Males		Females	
	0	0.1	0	0.1
PND 22				
Number of animals	23	20	22	21
Body weight (g)	47	43	48	40
Peak amplitude	41 ± 18	45 ± 14	47 ± 20	47 ± 14
Latency to peak	35 ± 6	39 ± 6	35 ± 6	42 ± 10
PND 38				
Number of animals	23	20	22	21
Body weight (g)	147	139	127	117
Peak amplitude	136 ± 63	191*** ± 68 (140)[b]	134 ± 90	154 ± 63 (115)
Latency to peak	31 ± 2	30 ± 2	32 ± 3	29 ± 2
PND 60				
Number of animals	23	20	22	21
Body weight (g)	283	279	192	181
Peak amplitude	349 ± 161	514*** ± 167 (147)	296 ± 160	351 ± 132 (119)
Latency to peak	34 ± 2	33 ± 1	35 ± 4	33 ± 2

[a] Data were obtained from pages 217-218, MRID 45441302.

[b] Number in parentheses is percent of control value calculated by reviewer.

*** Significantly different from controls at $p \leq 0.001$

TABLE 9b. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 male rats[a]

Water conc. (mg/mL)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 22						
0	Peak Amp.	42 ± 16	42 ± 19	44 ± 22	39 ± 22	35 ± 17
	Latency	38 ± 8	33 ± 6	33 ± 6	37 ± 9	35 ± 9
0.1	Peak Amp.	45 ± 14	45 ± 17	47 ± 17	44 ± 17	41 ± 14
	Latency	39 ± 8	39 ± 10	39 ± 9	37 ± 8	39 ± 9
PND 38						
0	Peak Amp.	159 ± 82	145 ± 63	147 ± 79	118 ± 66	112 ± 62
	Latency	31 ± 2	30 ± 3	30 ± 2	31 ± 3	31 ± 3
0.1	Peak Amp.	222***± 87 (140)[b]	219***± 81 (151)	192 ± 68 (131)	173***± 63 (147)	149 ± 67 (133)
	Latency	32 ± 3	29 ± 2	29 ± 2	30 ± 3	30 ± 3
PND 60						
0	Peak Amp.	452 ± 179	415 ± 195	332 ± 185	289 ± 171	256 ± 147
	Latency	36 ± 2	34 ± 3	34 ± 3	34 ± 2	34 ± 3
0.1	Peak Amp.	611***± 132 (135)	561***± 190 (135)	491*** ± 207 (148)	467***± 175 (162)	441*** ± 187 (172)
	Latency	34 ± 2	34 ± 2	33 ± 2	33 ± 2	33 ± 22

[a] Data were obtained from pages 220-222, MRID 45441302.

[b] Number in parentheses is percent of control value calculated by reviewer

N = 20-23

*** Significantly different from controls at $p \leq 0.001$

TABLE 9c. Mean (±SD) interval acoustic startle peak amplitude (g) and latency to peak (msec) in F_1 female rats[a]

Water conc. (mg/mL)	Parameter	Block 1	Block 2	Block 3	Block 4	Block 5
PND 22						
0	Peak Amp.	50 ± 19	51 ± 19	47 ± 24	45 ± 23	42 ± 23
	Latency	37 ± 7	34 ± 8	34 ± 9	35 ± 8	35 ± 7
0.1	Peak Amp.	45 ± 13	49 ± 14	51 ± 19	46 ± 17	45 ± 18
	Latency	46 ± 10	43 ± 11	41 ± 11	41 ± 10	40 ± 11
PND 38						
0	Peak Amp.	154 ± 105	153 ± 107	137 ± 101	119 ± 87	105 ± 66
	Latency	34 ± 4	31 ± 4	32 ± 4	32 ± 6	33 ± 6
0.1	Peak Amp.	159 ± 62	158 ± 67	161 ± 78	158 ± 68	135 ± 66
	Latency	31 ± 4	29 ± 3	29 ± 4	29 ± 4	28 ± 2
PND 60						
0	Peak Amp.	369 ± 190	377 ± 178	304 ± 176	238 ± 154	191 ± 154
	Latency	36 ± 4	35 ± 4	34 ± 5	34 ± 5	35 ± 6
0.1	Peak Amp.	425 ± 155	437 ± 163	372 ± 145	282 ± 144	242 ± 126
	Latency	34 ± 3	32 ± 2	33 ± 2	33 ± 3	32 ± 4

[a] Data were obtained from pages 223-225, MRID 45441302.

N = 21-22

c. <u>Learning and memory testing</u>: Passive avoidance data are presented in Table 10a. There were no treatment-related differences in the number of trials to criterion or the latency to cross for trials one and two on either test occasion.

Water maze data are included in Table 10b. The number of trials to criterion during both the learning and retention phases was higher for treated animals with the change being significant in males during the learning phase. Trial duration for trials one and two was also increased in treated animals; the change was significant in males during the learning phase. There were also more treated animals that failed to cross during the learning phase (two males and two females vs. one male and no females in the control group).

TABLE 10a. Passive avoidance performance data (mean ±SD) in F_1 rats [a]

Parameter	Water concentration (mg/mL)			
	Males		Females	
	0	0.1	0	0.1
Session 1 (Learning phase, PND 24)				
Number of animals tested	23	20	23	21
Number of animals included in analysis	22	17	23	20
Trials to criterion	3.1 ± 0.7	3.0 ± 0.6	3.6 ± 1.0	3.2 ± 0.9
Latency trial 1	58.5 ± 56.7	65.7 ± 62.6	39.5 ± 54.3	56.2 ± 62.4
Latency trial 2	171.0 ± 22.6	180.0 ± 0.0	168.4 ± 25.0	174.1 ± 26.5
Failed to meet criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Failed to cross during learning phase	3 (13%)	2 (10%)	2 (9%)	3 (14%)
Excluded from learning phase analyses	1 (4%)	3 (15%)	0 (0%)	1 (5%)
Session 2 (Retention phase, PND 31)				
Number of animals tested	23	20	23	21
Number of animals included in analysis	19	12	21	16
Trials to criterion	2.4 ± 0.8	2.6 ± 0.9	2.4 ± 0.7	2.4 ± 0.7
Latency trial 1	166.3 ± 38.7	167.5 ± 32.0	152.8 ± 54.6	158.0 ± 54.9
Latency trial 2	168.1 ± 36.7	158.9 ± 45.6	173.5 ± 26.9	169.6 ± 29.6
Excluded from retention phase analyses	0 (0%)	4 (20%)	0 (0%)	1 (5%)

[a] Data were obtained from pages 227-228, MRID 45441302.
Trials to criterion = mean number of trials per group ± SD.
Latency to trial 1 = mean session 1 duration (seconds) per group ± SD.
Latency to trial 2 = means session 2 duration (seconds) per group ± SD.
Failed to meet criterion = number of animals that received the shock but did not demonstrate acquisition.
Failed to cross = number of animals that never received the shock.
Numbers in parenthesis indicate percentage of animals with specific finding, calculated by reviewer.

TABLE 10b. Watermaze performance data (mean ±SD) in F_1 rats [a]

Parameter	Water concentration (mg/mL)			
	Males		Females	
	0	0.1	0	0.1
Session 1 (Learning phase)				
Number of animals	23	20	23	21
Trials to criterion (mean ± S.D.)	7.3 ± 2.5	9.5* ± 3.3	8.1 ± 2.8	10.0 ± 3.0
Trial 1 – Errors (mean ± S.D.)	0.9 ± 0.9	0.9 ± 0.7	0.7 ± 0.7	0.7 ± 0.7
Trial 1 – Duration (sec) (mean ± S.D.)	18.4 ± 11.9	24.3 ± 17.6	16.1 ± 9.5	17.6 ± 11.9
Trial 2 – Errors (mean ± S.D.)	0.5 ± 0.8	0.8 ± 0.9	0.6 ± 1.1	1.0 ± 1.5
Trial 2 – Duration (sec) (mean ± S.D.)	15.0 ± 13.1	20.3* ± 12.0	14.0 ± 13.1	19.7 ± 15.9
Failed to meet criterion	1 (4%)	2 (10%)	0 (0%)	2 (10%)
Session 2 (Retention phase)				
Number of animals	22	18	23	19
Trials to criterion (mean ± S.D.)	5.8 ± 1.9	6.5 ± 2.3	8.2 ± 3.7	9.5 ± 4.4
Trial 1 – Errors (mean ± S.D.)	0.2 ± 0.4	0.8 ± 1.2	0.6 ± 1.0	0.5 ± 0.9
Trial 1 – Duration (sec) (mean ± S.D.)	9.6 ± 11.7	17.3 ± 18.2	10.3 ± 9.7	12.1 ± 10.3
Trial 2 – Errors (mean ± S.D.)	0.0 ± 0.0	0.2 ± 0.4	0.1 ± 0.5	0.3 ± 0.6
Trial 2 – Duration (sec) (mean ± S.D.)	4.2 ± 1.7	7.8 ± 6.7	6.6 ± 5.0	8.1 ± 5.8

[a]Data were obtained from pages 230-231, MRID 45441302.
*Significantly different from controls at $p \leq 0.05$
Numbers in parenthesis indicate percentage of animals with specific finding, calculated by reviewer.

5. **Ophthalmology:** No treatment-related lesions were observed.

6. **Thyroid Evaluations**: The results of the thyroid function tests and thyroid weight at post-mortem are presented in Table 11. On PND 11, T_4 levels were significantly decreased (16% of control value) in treated males and females. T_3 levels were significantly decreased in treated males (82% of control value) and non-significantly decreased in treated females (86% of control value). On PND 70, T_3 and T_4 levels were increased (119% of control value) in treated males. T_4 and T_3 levels in treated females were comparable to control values on PND 70. Absolute thyroid weight was unaffected by treatment on PND 70. Relative thyroid weight was slightly increased in treated males and females due to decreased terminal body weight in both sexes. On microscopic examination, 12/12 treated males and 9/9 treated females had hypertrophy/hyperplasia of the thyroid compared to none in the control group. There were no treatment-related microscopic effects at the PND 70 necropsy. No treatment-related effects were found in the immunohistochemistry evaluations on PNDs 11 and 70.

TABLE 11. Thyroid function tests and postmortem weight (mean ±SD) in F_1 rats [a]				
Parameter	**Water concentration (mg/mL)**			
	Males		**Females**	
	0	**0.1**	**0**	**0.1**
Day 11				
T_4 (µg/dL)	4.04 ± 0.77	0.66* ± 0.44 (16)[b]	4.34 ± 0.37	0.69* ± 0.35 (16)
T_3 (µg/dL)	0.57 ± 0.08	0.47* ± 0.10 (82)	0.58 ± 0.06	0.50 ± 0.12 (86)
Day 70				
T_4 (µg/dL)	4.83 ± 0.44	5.76* ± 0.85 (119)	3.92 ± 1.25	3.26 ± 0.88 (83)
T_3 (µg/dL)	0.62 ± 0.13	0.74 ± 0.16 (119)	0.57 ± 0.15	0.45 ± 0.11 (79)
Terminal body weight (g)	331.0 ± 17.2	303.4* ± 21.2 (92)	214.6 ± 13.3	199.0* ± 7.7 (93)
Thyroid weight (g)	0.020 ± 0.003	0.021 ± 0.004	0.018 ± 0.003	0.018 ± 0.004
Thyroid-to-body weight ratio (%)	0.006 ± 0.001	0.007* ± 0.001 (117)	0.008 ± 0.001	0.009 ± 0.002 (113)

[a] Data were obtained from pages 640-644 and 668-669, MRID 45441302.
[b] Number in parentheses is percent of control; calculated by reviewer.
N = 10-12 for Day 11; 10 for Day 70.
* Significantly different from control group, $p<0.05$

7. Postmortem results:

a. Brain weights and measurements: Mean brain weight and gross measurement data are presented in Table 12. On PND 11, absolute brain weight was significantly decreased (91-92% of control value) in treated males and females. Correspondingly, the length of the cerebrum was significantly reduced in treated males and females on PND 11. On PND 70, absolute brain weight and the length of the cerebrum and cerebellum were unaffected by treatment.

TABLE 12. Mean (±SD) brain weight and measurement data [a]				
Parameter	**Water concentration (mg/mL)**			
	Males		**Females**	
	0	**0.1**	**0**	**0.1**
Day 11				
Brain weight (g)	1.17 ± 0.12	1.06* ± 0.11 (91)[b]	1.15 ± 0.06	1.06* ± 0.09 (92)
Cerebrum length (mm)	12.48 ± 0.54	11.94* ± 0.56 (96)	12.40 ± 0.20	12.10* ± 0.41 (98)
Cerebellum length (mm)	7.22 ± 0.37	7.22 ± 0.26	7.25 ± 0.35	7.20 ± 0.51
Day 70				
Brain weight (g)	1.91 ± 0.06	1.88 ± 0.08	1.75 ± 0.09	1.75 ± 0.07
Cerebrum length (mm)	14.86 ± 0.23	14.96 ± 0.35	14.43 ± 0.20	14.26 ± 0.38
Cerebellum length (mm)	7.29 ± 0.24	7.28 ± 0.13	6.89 ± 0.27	7.12 ± 0.29

[a] Data obtained from pages 668-672 in the study report.
N = 10-12 for Day 11; 16 for Day 70.
[b] Number in parentheses is percent of control; calculated by reviewer.
*Significantly different from controls at $p\leq 0.05$

b. Neuropathology:

1.) Macroscopic examination: No treatment-related lesions were observed.

2.) **Microscopic examination:** Histopathology data are included in Table 13. On PND 11, treated male and female pups had treatment-related increased morphological changes or mean severity increases in brain lesions. The incidence of oligodendroglia prominence (increase in number of oligodendrogial cells) was higher or the severity of the lesions was increased for all 8 brain levels in treated males and in levels 2-8 in treated females. The other treatment-related finding was neuropil maturation (neurons appear closer together with not as much neuropil separating the cells). The incidence of this finding was increased in treated males (levels 2-6) and females (levels 3-6). No microscopic lesions were observed at the PND 70 necropsy.

TABLE 13. Histopathology findings on PND 11 – number affected (severity) [a]

Parameter	Water concentration (mg/mL)			
	Males		Females	
	0	0.1	0	0.1
Brain, Level 1				
Number tissues examined	12	10	10	11
No abnormality detected	12	8	10	11
Tissue missing	-	-	1	-
Oligodendroglia prominence	-	2 (2.0)	-	-
Brain, Level 2				
Number tissues examined	12	10	11	11
No abnormality detected	2	-	1	-
Neuropil maturation	-	2 (2.0)	-	-
Oligodendroglia Prominence	10 (1.3)	10 (2.6)	10 (1.4)	11 (1.9)
Brain, Level 3				
Number tissues examined	12	10	11	11
No abnormality detected	2	-	1	-
Neuropil maturation	-	5* (2.0)	-	2 (2.0)
Oligodendroglia prominence	10 (1.1)	10 (2.7)	10 (1.1)	11 (2.5)
Brain, Level 4				
Number tissues examined	12	10	11	11
No abnormality detected	1	-	1	-
Neuropil maturation	1 (1.0)	7* (1.9)	1 (1.0)	3 (2.0)
Oligodendroglia prominence	11 (1.1)	10 (2.7)	10 (1.2)	11 (2.5)
Brain, Level 5				
Number tissues examined	12	10	11	11
No abnormality detected	1	-	1	-
Neuropil maturation	1 (2.0)	8* (1.8)	2 (1.0)	8* (2.0)
Oligodendroglia prominence	11 (1.2)	9 (2.9)	10 (1.3)	11 (2.5)
Brain, Level 6				
Number tissues examined	12	10	11	11
No abnormality detected	2	1	-	1
Neuropil maturation	-	6* (1.8)	-	2 (2.0)
Oligodendroglia prominence	10 (1.1)	9 (2.7)	11 (1.3)	10 (2.5)
Brain, Level 7				
Number tissues examined	12	10	11	11
No abnormality detected	3	-	-	1
Oligodendroglia prominence	9 (1.3)	10 (2.2)	11 (1.2)	10 (1.9)
Brain, Level 8				
Number tissues examined	12	10	11	11
No abnormality detected	10	5	3	4
Oligodendroglia prominence	2 (1.0)	5 (1.8)	8 (1.4)	7 (1.6)

[a] Data obtained from pages 689-690, MRID 45441302.

* Significantly different from control, $p<0.05$.

Level 1 = olfactory bulb section; Level 2 = olfactory region section; Level 3 = forebrain (optic nerve) section; Level 4 = forebrain (optic chiasm) section; Level 5 = midbrain section; Level 6 = mesencephalon section; Level 7 = cerebellum/pons section; Level 8 = cerebellum/medulla oblongata section

3.) <u>Brain Morphometry</u>: Morphometric data for PNDs 11 and 70 are presented in Table 14.

On PND 13, the length of the cerebrum was significantly decreased (96-98% of control value) in both sexes of treated animals. No treatment-related differences in cerebellum length were observed. Measurements of the frontal cortex, parietal cortex, caudate putamen and hippocampal gyrus were significantly decreased in treated males on PND 11. The only significant change on PND 70 was an increase in length of the parietal cortex in treated females.

TABLE 14. Mean (±SD) morphometric measurements (mm) in perfused F_1 rats on PND 11[a]

Parameter	Water concentration (mg/mL)			
	Males		Females	
	0	0.1	0	0.1
Day 11				
Frontal cortex (mm)	1.619 ± 0.044	1.341*** ± 0.009	1.471 ± 0.120	1.334 ± 0.007
Caudate putamen (mm)	2.715 ± 0.035	2.436*** ± 0.029	2.555 ± 0.016	2.421 ± 0.015
Parietal cortex (mm)	1.661 ± 0.026	1.477** ± 0.006	1.523 ± 0.014	1.444 ± 0.005
Corpus callosum (mm)	0.685 ± 0.038	0.593 ± 0.004	0.556 ± 0.013	0.527 ± 0.009
Hippocampal gyrus (mm)	1.300 ± 0.016	1.155** ± 0.004	1.205 ± 0.019	1.130 ± 0.007
External germinal layer (mm)	0.083 ± 0.000	0.087 ± 0.000	0.084 ± 0.000	0.088 ± 0.000
Cerebellum (mm)	4.376 ± 0.264	3.940 ± 0.267	4.505 ± 0.256	4.073 ± .204
Day 70				
Frontal cortex (mm)	1.929 ± 0.033	1.907 ± 0.005	1.885 ± 0.004	1.824 ± 0.014
Caudate putamen (mm)	3.773 ± 0.061	3.705 ± 0.036	3.676 ± 0.032	3.656 ± 0.038
Parietal cortex (mm)	2.179 ± 0.004	2.222 ± 0.005	2.083 ± 0.000	2.221*** ± 0.005
Corpus callosum (mm)	0.456 ± 0.006	0.493 ± 0.002	0.426 ± 0.001	0.456 ± 0.001
Hippocampal gyrus (mm)	1.998 ± 0.025	2.043 ± 0.056	1.945 ± 0.016	1.931 ± 0.011
Cerebellum (mm)	5.953 ± 0.132	5.949 ± 0.028	5.690 ± 0.008	5.862 ± 0.028

[a] Data obtained from pages 674-682, MRID 45441302.
** Significantly different from control, $p<0.01$
*** Significantly different from control, $p<0.001$

III. DISCUSSION and CONCLUSIONS:

A. <u>INVESTIGATORS' CONCLUSIONS</u>: The study author concluded that the study provided evidence of a variety of neurobehavioral, morphologic and hormonal effects associated with perinatal exposure to methimazole consistent with the known anti-thyroid effects of the chemical. The study determined the reproducibility of results for tests that constitute the DNT testing battery, the reliability of equipment operation and the consistency of results across devices and across days for any one device as well as providing data that demonstrate the laboratory's competence in evaluating effects in neonatal animals perinatally exposed to chemicals.

B. <u>REVIEWER COMMENTS</u>: The reviewer agrees that the laboratory generally demonstrated competence in testing for developmental neurotoxicity in offspring from treated dams. Although some effects could be attributed to decreased body weight in pups from treated dams, the methods were well described and individual animal data were

included in the report. Brain weight, gross measurements, neuropathology, and morphometry results were well correlated for the effects seen in PND 11 offspring. Effects on motor activity and differences in brain morphometry observed in pre-weaning rats had resolved by study termination indicating that the laboratory was capable of detecting changes in young animals. Positive findings during the FOB would have been useful in evaluating the laboratory's ability to perform this test in various ages of rat. It is suggested that the laboratory submit these data for the FOB. Use of only one dose precluded evaluation of a dose-response.

The significance of the results for males during auditory startle habituation testing is unknown. An increase in the peak amplitude was observed for treated males on PNDs 38 and 60 despite a lower body weight for these animals. These results suggest an enhanced response to the startle stimulus, although lack of additional dose levels prevents a dose-response assessment.

Bayer Corporation has demonstrated proficiency in this study for detecting changes in Auditory Startle, Motor Activity, Thyroid Function, and Brain Neuropathology and Morphometry in pre- and postweaning and young adult (depending on endpoint) Wistar Crl:WI(HAN)BR rats due to methimazole treatment for the time period around 1998-1999 (in life period of study).

C. STUDY DEFICIENCIES:

1. No analytical data were submitted to demonstrate that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

2. Analyses of the test material in the drinking water were not included in the study report.

3. The Methods section of the study report does not adequately describe how some of the testing was conducted, including FOB in dams and offspring, ophthalmology and thyroid immunohistochemistry.

4. A dose-response could not be evaluated.

The deficiencies did not affect the acceptability of the study because it was designed as a positive control study.